

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
12 July 2001 (12.07.2001)

PCT

(10) International Publication Number
WO 01/49282 A2

(51) International Patent Classification⁷:

A61K 31/00

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(21) International Application Number:

PCT/US01/00507

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(22) International Filing Date:

8 January 2001 (08.01.2001)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/174,708 6 January 2000 (06.01.2000) US

Published:

— Without international search report and to be republished upon receipt of that report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(71) Applicant (for all designated States except US):

MARTEK BIOSCIENCES CORPORATION [US/US];
6480 Dobbin Road, Columbia, MD 21045 (US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **KYLE, David, J.**
[US/US]; 1801 Narbeth Road, Catonsville, MA 21228 (US).

(74) Agents: **POSORSKE, Laurence, H. et al.**; Brobeck, Phleger & Harrison LLP, Intellectual Property Department, 1333 H Street, N.W., Suite 800, Washington, DC (US).

(54) Title: THERAPEUTIC PREPARATIONS OF HIGHLY UNSATURATED FATTY ACIDS

WO 01/49282 A2

(57) Abstract: This invention provides a therapeutic composition comprising highly unsaturated fatty acids (HUFAs) in an amount and form sufficient to produce normal or supernormal levels of one or more of the respective HUFAs in one or more tissues of an individual in need thereof upon administration of the composition. Preferably, administration of this composition will raise the tissue level of one or more HUFA to over twice normal in a target tissue, more preferably, the tissue level of DHA is raised to over twice normal in the target tissue. Typically, the individual in need of the therapeutic composition is a mammal, especially a human, suffering from a disease selected from the group consisting of cystic fibrosis, multiple sclerosis, cerebral palsy, amyotrophic lateral sclerosis, phenylketonuria, and neurological disorders, including certain neurodegenerative diseases and psychiatric disorders. Preferably, the levels of one or more of the respective HUFAs, especially DHA, are normalized or supernormalized in membranes of the individual. In another preferred mode of this invention, the HUFAs in the therapeutic composition are in a readily absorbable form, such that the dose of HUFA required to achieve normal or supernormal levels in the individual is lower than the dose required for HUFA-containing triglycerides. For example, the HUFAs may be predominantly in the form of glycerol esters. Preferably at least 70% of the HUFAs are esterified to glycerol; more preferably, the HUFAs are predominantly in the form of diglycerides and/or monoglycerides.

THERAPEUTIC PREPARATIONS OF HIGHLY UNSATURATED FATTY ACIDS**BACKGROUND****Field of the Invention**

5 This invention relates to methods of treating pathologies which are characterized by abnormally low levels of docosahexaenoic acid and other highly unsaturated fatty acids (HUFA) in characteristic tissues using a preparation of an absorbable form of the HUFA, especially docosahexaenoic acid (DHA) with little or no accompanying eicosapentaenoic acid (EPA), such that the oral consumption of
10 such a preparation leads to the normalization or supernormalization of the tissue HUFA (e.g., DHA) levels and reduction of the pathologic symptoms. In particular, this invention relates to a method of treating cystic fibrosis, multiple sclerosis, cerebral palsy, amyotrophic lateral sclerosis, phenylketonuria, and neurological disorders, including certain neurodegenerative diseases and psychiatric disorders, by
15 administering a composition comprising a therapeutically effective amount of a HUFA-rich triglyceride oil, wherein the triglyceride is hydrolyzed or pre-digested prior to consumption.

Review of the Related Art

20 Docosahexaenoic acid (4, 7, 10, 13, 16, 19-docosahexaenoic acid 22:6, ω -3) (hereinafter referred to as DHA) is a long chain polyunsaturated fatty acid which is thought to play an important role in modulating the structure, fluidity and function of the cell membranes of tissues such as pancreas, intestine, lung, neural tissues, and retina. DHA cannot be synthesized *de novo* in humans, but there is some evidence that this ω -3 fatty acid can be synthesized by some cell types, such as
25 astrocytes, if the appropriate long chain polyunsaturated fatty acids are provided in the diet. S. Moore, et al., 1991, *J. Neurochem.*, **56**:518-524. Most of the DHA found in cell membranes is believed to be obtained from dietary sources. Other HUFA are also difficult for humans to synthesize, and dietary sources are important for maintaining adequate levels of the respective HUFAs in tissues.

Because of the importance of DHA in cell membrane structure, fluidity, and function, hypodocosahexaenemia is believed to have a causal relationship to the pathological symptoms of many disorders. Additionally, many disorders are thought to result in low DHA levels. The role of HUFA levels for 5 good health and in disease prevention and treatment is further discussed in International Patent Publication WO 96/40106.

Many disorders that result in low tissue DHA levels may also affect pancreatic function and therefore normal digestion. Cystic fibrosis (CF), for example, is a disease resulting in low tissue levels of DHA in the pancreas, intestine, 10 and lung, and one consequence of this disease is the inability of an individual to digest fat (triglycerides) very efficiently.

Where DHA is associated with disease pathology, the elevation of tissue DHA levels may be sufficient to convert the abnormal tissue structure (the pathology) to a normal tissue structure. Freedman et al (1999, *Proc. Natl. Acad. Sci. 15 USA*, **96**:13995, incorporated herein by reference) reported the treatment of a mouse model of cystic fibrosis (CFTR(-/-) mouse) with free or esterified DHA at very high concentrations (40mg/mouse/day) for 7 days could effectively normalize the tissue pathology. Lower concentrations were not effective at altering the pathology. A dose of 40 mg DHA/mouse/day resulted in an increase in circulating DHA to levels 20 of about 250 µg DHA/ml. Over-the-counter preparations of DHA can be potentially difficult to use in humans since only very large amounts proved to be effective in mice (40 mg/mouse/day is equivalent to about 2000 mg/kg/day or 130 g DHA/person/day). Based on these results, it appears that the nutritional approach to treating CF and other pathologies characterized by abnormally low levels of 25 docosahexaenoic acid in characteristic membranes would not be effective.

Freedman et al (1999) demonstrated that the phenotypic expression of CF in the (CFTR(-/-)) mouse model could be prevented if the mouse was supplied with large amounts (40 mg/mouse/day) of DHA. However, in the mouse model, the

protein associated with CF (CF transmembrane conductance regulator or CFTR) is absent. In contrast, the CFTR protein is present but modified in the human condition, not completely absent as in the knock-out mouse model. It is not apparent that changes in the lipid membrane in a system where a protein is absent would predict similar responses when the protein is present, but modified (as in the human condition). The differences between the mouse model (protein absent) and the human disease (protein present but modified) prevent clear extrapolation from the model to the human. In fact, there have been several attempts to affect CF disease by the treatment with oral fish oil (a source of DHA, as well as other fatty acids), but these have lead to no statistically significant improvements between control patients and patients treated with fish oil. Based on these observations, the use of sources of DHA in a dietary mode for the prevention of the CF pathology has not appeared to be a promising avenue for research.

In addition to the lack of success of experiments attempting to treat CF using oral fish oil, these experiments are also problematic because they provide DHA accompanied by large amounts of EPA. It has also been reported that marine oil capsules rich in both EPA and DHA raise LDL-cholesterol levels (the fraction of serum cholesterol that is the most atherogenic), particularly in patients with Type IIb and IV dyslipidemia (Davids, et al., 1991, "Therapy for the Treatment of Hyperlipidemia," *Archives of Internal Medicine*, **151**:1732-1740; Harris, et al., 1988, "Effects of a Low Saturated Fat, Low Cholesterol Fish Oil Supplement in Hypertriglyceridemia Subjects," *Ann. Intern. Med.*, **109**:465-470). In view of the effect of marine oils on cholesterol metabolism, clinical enthusiasm for the use of marine oil capsules has been dampened. Accordingly, there is a need for therapy having the beneficial effects of DHA without the side effects of raising cholesterol.

SUMMARY OF THE INVENTION

It is an object of this invention to provide inexpensive, dietary methods of treatment for pathologies, such as cystic fibrosis, that are characterized

by abnormally low levels of HUFA, such as docosahexaenoic acid, in the membrane lipids of characteristic tissues.

It is an object of this invention to provide methods of treating pathologies which are characterized by abnormally low levels of docosahexaenoic acid in the membrane lipids of characteristic tissues using a preparation of an absorbable form of docosahexaenoic acid (DHA) with little or no accompanying eicosapentaenoic acid (EPA), such that the oral consumption of such a preparation leads to the normalization or supernormalization of the tissue DHA levels and reduction of the pathologic symptoms. This and other objects are met by one or 10 more of the following embodiments.

In one embodiment, a disorder selected from the group consisting of cystic fibrosis, multiple sclerosis, cerebral palsy, amyotrophic lateral sclerosis, phenylketonuria, and neurological disorders, including certain neurodegenerative diseases and psychiatric disorders, is treated by administration of a preparation of a 15 readily absorbable form of one or more HUFAs, preferably including docosahexaenoic acid (DHA) with little or no accompanying eicosapentaenoic acid (EPA), such that the oral consumption of such a preparation leads to the normalization or supernormalization of the tissue levels of the respective HUFAs and reduction of the pathologic symptoms.

20 In a preferred embodiment, the preparation comprises a therapeutically effective amount of a DHA-rich triglyceride oil, wherein the triglyceride is hydrolyzed or pre-digested prior to consumption to yield DHA completely in the form of monoglycerides, completely in the form of diglycerides, completely in the form of free fatty acids, or any combinations or mixtures thereof.

25 In another embodiment, the preparation comprises a therapeutically effective amount of a HUFA-rich oil, wherein one or more HUFAs are in the form of triglycerides, diglycerides, monoglycerides, or free fatty acids, or any combinations or mixtures thereof.

In an alternative embodiment, the preparation comprises a therapeutically effective amount of a HUFA-rich triglyceride oil, wherein the triglyceride is synthesized directly from pure HUFA, such as DHA, and glycerol. In a more preferred embodiment, the preparation comprises a therapeutically effective amount of DHA, wherein the DHA is in the form of triglycerides, diglycerides, monoglycerides, or free fatty acids, or any combinations or mixtures thereof, wherein the tri-, di- and mono- glycerides or a portion thereof are synthesized directly from pure DHA and glycerol.

In another embodiment, the preparation comprises a therapeutically effective amount of a HUFA-rich triglyceride oil and enzymes that hydrolyze triglycerides, wherein said enzymes are suitable for human or mammalian consumption. In yet another embodiment, the preparation comprising a therapeutically effective amount of a HUFA-rich triglyceride oil is co-administered with enzymes that hydrolyze triglycerides, wherein said enzymes are suitable for human or mammalian consumption. Preferably the HUFA-rich oil is a DHA-rich oil.

In another important embodiment, the preparation contains little or no EPA (less than 1 part EPA to 3 parts DHA, more preferably less than 1 part EPA to 5 parts DHA). In a preferred embodiment, the preparation contains less than 20% EPA. In a more preferred embodiment, the preparation contains less than 10% EPA. In a still more preferred embodiment, the preparation contains less than 5% EPA.

In any of the foregoing embodiments, the oil may be hydrolyzed completely to provide HUFAs, such as DHA, as free fatty acids. The oil may be hydrolyzed partially, providing HUFAs in a form of mixed free fatty acids, monoglycerides, diglycerides, and/or triglycerides. The preparation may comprise HUFAs, such as DHA, completely in the form of triglycerides, completely in the form of diglycerides, completely in the form of monoglycerides, completely in the form of free fatty acids, or as mixtures thereof.

In any of the foregoing embodiments, the HUFA-rich triglyceride oil is preferably pre-digested. Hydrolysis of the oil may be performed by treatment of the HUFA-rich triglyceride with enzymes such as, but not limited to, pancreatic lipase. Alternatively, the HUFA-rich triglyceride may be chemically hydrolyzed. In 5 another alternative, the oil may be subject to glycerolysis, optionally with enzyme catalysis. As yet another alternative, the oil can be partially saponified to yield mixed glycerides and free fatty acids. Preferably, the resulting oil predominantly comprises diglycerides, monoglycerides, free fatty acids, or combinations thereof.

Purified, free HUFA, such as DHA, can also be added to the 10 hydrolysis or pre-digestion mixture for the enrichment of the glycerides with the HUFA. This process will produce a mixture that is more highly enriched in the HUFA and preferable for the treatment of the disease. Depending on the conditions under which the added, purified HUFA is incubated in the hydrolysis or pre-digestion mixture, the added DHA may remain in the free acid form or may be at 15 least partially esterified to glycerol.

In one important embodiment, the hydrolysis or pre-digestion is performed on an oil in which the DHA content is above 20% such as tuna fish oil. In a more preferred embodiment, the hydrolysis or pre-digestion is performed on an oil in which the DHA content is over 40% DHA, such as the oil produced by the alga 20 *Cryptocodonium cohnii* or by fungi of the chytrid family (e.g., *Thraustochytrium*).

In any of the foregoing embodiments, DHA in the form of triglycerides may be obtained as oil in which the DHA content is above 20% such as tuna fish oil. More preferably, in any of the foregoing embodiments, DHA in the form of triglycerides may be obtained as oil in which the DHA content is over 40% 25 DHA, such as the oil produced by the alga *Cryptocodonium cohnii* or by fungi of the chytrid family (e.g., *Thraustochytrium*). DHA supplementation is a preferred mode of this invention; however, supplementation or therapy with glycerol esters

of other HUFAs, such as arachidonic acid (ARA), is also within the contemplation of this invention, either as the sole supplementary HUFA, or in combination with DHA.

Oils containing the respective HUFAs can be administered as a pharmaceutical composition, as a dietary supplement, or in the form of a food product by replacing a portion of the vegetable oil or fat normally found therein. Administration of these compositions provides prophylactic, as well as therapeutic, treatment of pathologies which are characterized by abnormally low levels of HUFAs in specific tissues, such as low docosahexaenoic acid in the membrane lipids of characteristic tissues, and of the symptoms associated with these pathologies.

Unexpectedly, much lower doses than used in the prior art are effective in humans when the DHA or other HUFA is provided in a form that is easily absorbed. If DHA is provided to the patient at doses of 5-20 g DHA/day (about one-tenth that predicted from the mouse model), the DHA content of the CF tissue increases dramatically and the pathology can be reversed. By returning the tissue levels of DHA to normal or even supernormal (preferably at least up to 3 times starting levels), some of the pathology associated with cystic fibrosis may be prevented. This represents a novel, inexpensive, dietary treatment for a disease such as cystic fibrosis.

DETAILED DESCRIPTION OF THE INVENTION

DHA is provided to the patient in a form that contains less than 1 part EPA for every 3 parts of DHA.

In accordance with this invention, DHA-rich oils are administered to patients affected a pathology characterized by depressed levels of DHA in the blood or tissues in comparison to the levels found in healthy individuals. Hypodocosahexaenemia may be found in particular tissues characteristic of certain diseases. For example, depression of DHA levels in cardiac or neuronal tissues, particularly in the cells having excitable membranes, can lead to pathology. As another example, cystic fibrosis is a disease resulting in low tissue levels of DHA in

the pancreas, intestine, and lung, and one consequence of this disease is the inability of an individual to digest fat (triglycerides) very efficiently.

The method of this invention includes administration of DHA or other HUFA to patients affected by disorders where depressed levels of the respective HUFA are characteristic of the disease. The therapeutic compounds of this invention are also useful for improving the condition of patients suffering as a result of inborn errors of metabolism which result, among other things, in depression of DHA levels in one or more tissues. Examples of diseases which may be treated according to the methods of this invention include multiple sclerosis, cerebral palsy, amyotrophic lateral sclerosis, phenylketonuria, cystic fibrosis, and neurological disorders, including certain neurodegenerative diseases and psychiatric disorders. By increasing the tissue levels of DHA and/or other HUFA to normal or even supernormal levels, at least some of the pathology associated with these diseases may be prevented, reduced, or reversed.

Examples of neurological disorders that may be treated with the methods according to this invention include Alzheimer's disease, Huntington's disease, schizophrenia, diabetic neuropathy, heavy metal toxicity. Other disorders which may be treated using the methods of the present invention include peroxisomal disorders, such as Zellweger's syndrome, neonatal adrenoleukodystrophy, infantile Refsum disease, hyperpepecolic acidemia, Rhizomelic chondrodysplasia punctata, Zellweger-like syndrome, adrenoleukodystrophy, adrenomyeloneuropathy, acyl-CoA oxidase deficiency, bifunctional protein deficiency, thiolase deficiency, hyperoxaluria type I, acatalasaemia and adult Refsum disease

The administration of DHA and other HUFAs to treat disorders is also described in patent publications WO 94/28913 and WO 96/40106, the texts of which patent publications are herein incorporated by reference.

DHA or other HUFA may be administered in a manner that results in increasing the level of the respective HUFA in the tissues where depressed levels of HUFA, such as DHA, contribute to the pathology of the disorder (hereinafter the "target" tissues or "characteristic" tissues). Appropriate target tissues may be 5 identified based on the abnormal or pathologic functional characteristics exhibited by the tissues in conjunction with depressed levels of DHA. Deviations from normal will be readily recognized by the skilled clinician familiar with the pathologic characteristics of particular diseases.

Preferably, the respective HUFA is administered in a therapeutically 10 effective amount, which is an amount the administration of which results in some therapeutic benefit for the patient. Such benefit can be, for example, a reduction in severity or frequency of occurrence of a symptom from which the patient suffers.

By increasing the tissue levels of DHA or other HUFA to normal or even supernormal (up to three times starting levels, for example), some of the 15 pathology associated with cystic fibrosis and other diseases characterized by hypodocosahexaenemia may be prevented, reduced or reversed. The tissue levels of DHA may be increased to between 2 and 5 times the levels for that tissue found in a subject without the pathology of interest. This increase is called "supernormalization."

20 However, as hypodocosahexaenemia may result in or be associated with an inability to digest fats efficiently, providing oral DHA as a triglyceride may not be very effective in treating the hypodocosahexaenemia. Providing the dose of DHA in a form, which is absorbable, or already pre-digested, facilitates the uptake 25 and incorporation into tissues. The provision of DHA or other HUFA in a form which is easily absorbable may also facilitate the uptake and incorporation into tissues of HUFA by patients who do not suffer from inability to digest fats efficiently.

The present inventor has discovered that when the DHA is provided in a pure and readily absorbable form, much lower doses are required than would be expected from the published mouse data.

Preferably, DHA is provided to the patient at doses of 5-20 g
5 DHA/day (about one-tenth that predicted from the mouse model). More preferably, DHA is provided at a level which triples the circulating DHA concentrations in a human patient.

A mixture of DHA or other HUFA in the form of diglycerides, monoglycerides, free fatty acids or combinations thereof may be obtained by
10 synthesizing these molecules from pure free fatty acids and glycerol.

More preferably, HUFA in the form of diglycerides, monoglycerides, free fatty acids, or combinations thereof may be obtained by hydrolyzing or pre-digesting HUFA-rich triglycerides. Hydrolysis may be performed by treatment of the HUFA-rich triglyceride with enzymes such as, but not limited to, esterases, 15 lipases, and other hydrolytic enzymes. For example, hydrolysis of the oil may be performed by treatment of DHA-rich triglyceride with pancreatic lipase. Alternatively, the DHA-rich triglyceride may be chemically hydrolyzed. In another alternative, the oil may be subject to glycerolysis, optionally with enzyme catalysis. As yet another alternative, the oil can be partially saponified to yield mixed 20 glycerides and free fatty acids.

Purified, free DHA or other HUFA can also be added to the hydrolysis mixture for the enrichment of the glycerides with the respective HUFA. This process will produce a mixture that is more highly enriched in DHA and preferable for the treatment of the disease. DHA in the form of purified, free fatty acids can also be added to the hydrolysis mixture, resulting in greater enrichment of the glycerides with DHA. Preferably, the resulting oil predominantly comprises diglycerides, monoglycerides, free fatty acids, or combinations thereof. Incubation
25

of HUFA-containing oil with free fatty acid during the hydrolysis or glycerolysis reaction will enrich the level of HUFA in the glycerol ester form.

5 DHA in the form of triglycerides may be obtained as oil in which the DHA content is above 20%. For example, DHA in the form of triglycerides may be obtained from tuna fish oil. More preferably, DHA in the form of triglycerides may be obtained from oil in which the DHA content is over 40% DHA.

Preferably, DHA in the form of triglycerides may be obtained as single cell microbial oils by the cultivation of DHA-producing microorganisms under oil-producing conditions.

10 Preferably, the preparation comprises a DHA-rich oil, which is an oil comprising at least 10% DHA. More preferably, the oil comprises at least 20% DHA. Even more preferably, the oil comprises at least 40% DHA. Most preferably, the oil comprises an even higher percentage of DHA, such as 60% or 80% DHA. Any of these oils may be referred to as a "DHA-rich oil" or a "DHA-containing oil."

15 Preferably, the preparation contains little or no EPA (less than 1 part EPA to 3 parts DHA). More preferably, the preparation contains less than 20% EPA. Still more preferably, the preparation contains less than 10% EPA. Most preferably, the preparation contains less than 5% EPA. A preparation containing such low levels of EPA is considered to have "little or no EPA." Particularly 20 preferred oils have fatty acids in which DHA is the only polyunsaturated fatty acid present in quantities greater than about 1% of the total amount of polyunsaturated fatty acids (PUFAs).

25 Therapy using oil rich in DHA and containing little or no EPA is superior to administration of fish oil. Increasing the absolute amount of a DHA taken up by the patient using fish oil inherently increases the amount administered of all the other fatty acids present in the oil. Thus, increasing the amount of fish oil administered to the patient will tend to increase the serum level of all HUFAs. In contrast, the preparations of this invention are high in DHA and contain little or no

EPA, thereby increasing uptake of DHA without increasing uptake of EPA and keeping the amount of EPA at the level which was present in the diet before initiation of therapy. More preferably, the oils used in the methods of the present invention contain very small amounts of or no highly unsaturated fatty acids 5 (HUFAs) other than DHA. Such an oil may be obtained, for example, from *C. cohnii*, as described above.

Provision of a preparation containing DHA and substantially free of EPA is important because individual HUFAs are processed via different pathways, and result in, for example, increased levels of different prostaglandins. Thus, excess 10 ARA can induce platelet aggregation (through prostaglandin PGE2 via the cyclooxygenase pathway) while excess EPA tends to reduce platelet aggregation. Omega-3 HUFA generally tend to have opposite effects to ω-6 HUFA.

Production of DHA-rich Oil

According to the preferred embodiments of the present invention, 15 microorganisms capable of producing a single cell microbial oil containing DHA are cultivated in a fermentor in a nutrient solution capable of supporting the growth of such organisms. Preferably, the microbial oil produced is enriched in DHA, meaning that it will contain at least about 20% DHA by weight. Methods for production of microbial oil containing DHA are described in WO 91/11918 and 20 corresponding National Phase applications.

Any microorganism capable of producing a microbial oil containing DHA can be used in the present invention. These microorganisms can be identified by determining whether DHA oil is present in the fatty acid profiles of the harvested biomass from a culture of the microorganism. These profiles are typically obtained 25 by gas chromatography of methyl ester derivatives of the fatty acids present in a sample.

As used herein, the term "microorganism," or any specific type of microorganism, includes wild-type strains, mutant strains or recombinant strains.

Wild-type and recombinant microorganisms designed to produce microbial cell oil containing DHA can be used to produce the DHA-rich microbial oil. Such recombinant strains would include those designed to produce greater quantities of DHA in the single cell oil, greater quantities of total oil, or both, as compared to the 5 quantities produced by the same wild-type microorganism, when provided with the same substrates. Microorganisms selected or designed to efficiently use more cost-effective substrates, while producing the same amount of single cell oil containing DHA as the wild-type microorganism, are particularly useful for preferred embodiments of the present invention.

10 For the production of DHA-rich microbial oils, species of photosynthetic algae, such as *Chattonella*, *Skeletonema*, *Thalassiosira*, *Isochrysis*, *Hymenomonas*, or *Cryptomonas* can be used. Preferred microorganisms are heterotrophic species of algae which include, but are not limited to, the Dinophyceae, for example, *Cryptocodonium*; or to fungi such as Chytridiomycetes, 15 for example, *Thraustochytrium*, *Schizochytrium* or *Ulkenia*, or the Oomycetes, for example, *Mortierella*, *Saprolegnia*, or *Mucor*.

20 Methods for obtaining DHA as single cell microbial oils via the culture of DHA-producing microorganisms are also detailed in U.S. Patent No. 5,492,938 to Kyle et al.; U.S. Patent No. 5,407,957 to Kyle, et al.; U.S. Patent No. 5,397,591 to Kyle et al.; U.S. Patent No. 5,130,242 to Barclay. Sources of other HUFA are described in the literature, including WO 92/13086 and WO 96/00182. The texts of these patents and applications are incorporated herein by reference.

Formulation, Doses, and Administration

25 The preparation may be provided as a triglyceride. However, in that embodiment, lipase enzymes, such as pancreatic lipase or pancreatin, are preferably also provided to digest the triglyceride before absorption. Preferably, the DHA is provided in an absorbable form (mixed glycerides and free fatty acid) to eliminate the need of additional enzymes to the patient.

The preparation may comprise a therapeutically effective amount of a DHA-rich triglyceride oil and enzymes that hydrolyze triglycerides, wherein said enzymes are suitable for human mammalian consumption. The preparation comprising a therapeutically effective amount of a DHA-rich triglyceride oil may be 5 co-administered with, i.e., administered substantially simultaneously with, enzymes that hydrolyze triglycerides, wherein said enzymes are suitable for human mammalian consumption. At least in these embodiments, the triglyceride is hydrolyzed or digested after consumption to yield DHA completely in the form of monoglycerides, completely in the form of diglycerides, completely in the form of 10 free fatty acids, or any combinations or mixtures thereof.

More preferably, the DHA-rich oil is administered in a readily absorbable form. In other words, the DHA-rich oil comprises DHA predominantly in the form diglycerides, monoglycerides, free fatty acids, or combinations or mixtures thereof. An oil which "predominantly comprises diglycerides, 15 monoglycerides, free fatty acids, or combinations thereof" is (1) an oil in which at least 50% of the triglycerides have been hydrolyzed or pre-digested to form diglycerides, monoglycerides, free fatty acids, or combinations thereof or (2) an oil formed from pure glycerol and pure DHA free fatty acids, in which less than 50% of the glycerides are triglycerides. As a short-hand, such an oil will be referred to as an 20 oil 50% free of triglycerides. Using this short-hand, more preferably, the DHA-rich oil is 60-70% free of triglycerides. Most preferably, the DHA-rich oil is 80% free of triglycerides. Any of these oils may be considered to be "DHA in a readily absorbable form." Oils administered to supply one or more other HUFAs, such as ARA, may be similarly treated to provide readily absorbable fatty acids.

25 The HUFA-rich oils can be administered as a pharmaceutical composition, as a dietary supplement, or in the form of a food product by replacing a portion of the vegetable oil or fat thereon. Administration of these compositions may provide prophylactic, as well as therapeutic, treatment of pathologies which are characterized by abnormally low levels of docosahexaenoic acid in the membrane

lipids of characteristic tissues and of the symptoms associated with these pathologies.

Although the HUFA-rich oils can be administered to patients directly, more commonly, they will be combined with one or more pharmaceutically or food acceptable carriers and, optionally, other therapeutic ingredients. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. Acceptable carriers are those which are compatible with the other components of the formulation and not deleterious to the patient. Carrier formulation suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations can be found in Remington's 5 Pharmaceutical Sciences, Mack Publishing Co., Easton, PA.

10 The HUFA-rich compositions of the present invention may be administered as a pharmaceutical composition. Preferably, they also may be formulated as a dietary supplement, such as a vitamin capsule or as food replacement 15 in the normal diet. Also preferably, they may be used as or added to food products.

Formulations include those suitable for oral, nasal, topical, or parenteral (including subcutaneous, intramuscular, intravenous, and intradermal) administration. It will be appreciate that the preferred formulation can vary with the 20 condition and age of the patient. The formulations conveniently can be presented in unit dosage form, e.g., emulsions, tablets, and sustained release capsules, and can be prepared by any suitable pharmaceutical method.

25 Formulations of the present invention suitable for oral administration can be presented as discrete units, such as capsules or tablets, each of which contains a predetermined amount of one or more HUFA, such as DHA. These oral formulations also can comprise a solution or a suspension in an aqueous liquid or a non-aqueous liquid. The solution can be an emulsion, such as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The oils can be administered by adding

the purified and sterilized liquids to a prepared enteral formula which is then placed into the feeding tube of a patient who is unable to swallow.

In one preferred embodiment, the HUFA-rich oil is incorporated into gel capsules. It will be recognized that any known means of producing gel capsules can be used in accordance with the present invention. Compressed tablets can be prepared by, for example, mixing the HUFA-rich oil with dry inert ingredients such as carboxymethyl cellulose and compressing or molding in a suitable machine. The tablets optionally can be coated or scored and can be formulated so as to provide slow or controlled release of the active ingredients therein.

Other formulations suitable for oral administration include lozenges comprising HUFA-rich oil in a flavored base, usually sucrose and acacia or tragacanth.

Formulations suitable for topical administration to the skin can be presented as ointments, creams and gels comprising the HUFA-rich oil in a pharmaceutically acceptable carrier. A preferred topical delivery system is a transdermal patch containing the oil to be administered.

In formulations suitable for nasal administration, the carrier is a liquid, such as those used in a conventional nasal spray or nasal drops.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which optionally can contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which can include suspending agents and thickening agents. The formulations can be presented in unit-dose or multi-dose containers. A preferred embodiment of the present invention includes incorporation of the HUFA-containing oil into a formulation for providing parenteral nutrition to a patient.

The DHA or other HUFA may also be incorporated into food products. The HUFA-rich oils can be administered as a cooking oil replacement

5 formulated so that in normal usage the patient would receive amounts of the respective HUFA sufficient to elevate the concentrations of this fatty acids in the serum and in membranes of target tissues to normal or supernormal levels. A special emulsion type margarine could also be formulated to replace butter or ordinary margarine in the diet. The HUFA-rich oils could be added to processed foods to provide an improved source of the respective HUFA. The oil can be microencapsulated using gelatin, casein, or other suitable proteins using methods known in the art, thereby providing a dry ingredient form of the oil for food processing.

10 It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention can include other suitable agents such as flavoring agents, preservatives and antioxidants. In particular, it is desirable to mix the microbial oils with an antioxidant to prevent oxidation of the DHA or other HUFA. Such antioxidants would be food acceptable and could include 15 vitamin E, carotene, BHT, ascorbyl palmitate or other antioxidants known to those of skill in the art.

Formulations and dosing are also described in patent publications WO 94/28913 and WO 96/40106, the texts of which patent publications are herein incorporated by reference.

20 While treatment is preferably monitored for dose adjustment by following the level of the respective HUFA in the target tissue, the skilled clinician may alternatively monitor the level of HUFA in a surrogate tissue, such as blood. Blood is a preferred surrogate tissue because it is relatively easy to obtain samples throughout treatment and monitor, e.g., DHA levels. DHA levels can also be 25 monitored in fractions of whole blood, such as serum, plasma, erythrocytes, etc. The specific course of treatment administered can be determined based on normalization or supernormalization of serum and erythrocyte HUFA levels. These serum levels of DHA are thought to reflect the long-chain polyunsaturated fatty-acid compositions

of membranes of other tissues. In some cases, serum levels of DHA may need to be increased to 2 to 5 times the levels which are considered to be normal in the general population in order to see a therapeutic effect.

The course of treatment can be followed by measuring levels of 5 HUFA in the serum of treated patients. For some patients, it will be possible to follow the normalization of DHA levels in neural tissue by measuring the levels of DHA in erythrocytes or in serum lipids during treatment. However, it is also recognized that DHA in target tissues may increase without a concomitant increase in blood levels of DHA. In this case, progress may be monitored by reference to 10 effects on pathologies and symptoms in addition to, or in place of, direct monitoring of the target tissues.

The daily dose of the compositions of the present invention to be provided to a patient may depend upon the weight of the patient and the extent of the DHA or other HUFA deficit identified by serum lipid analysis prior to the 15 introduction of the therapy. Additionally, the initial dose provided to a patient may differ from the maintenance dose. Also, the dosing may be altered based on the results the monitoring of DHA or other HUFA levels of the patient described herein. DHA may be provided to the patient at doses of from 1 to 50 grams DHA per day. Preferably, DHA may be provided to the patient at doses of from 5 to 30 grams per 20 day. More preferably, DHA is provided to the patient at doses of from 5 to 20 grams DHA per day. It will be appreciated that the amount of oil required to be provided to the patient to provide the desired doses of DHA will depend upon the degree to which the oil is enriched with DHA.

Desirably, the patient's HUFA profile is reviewed after about four 25 weeks of therapy. Subsequent doses then can be modified in response to the observed level of plasma lipid or red blood cell HUFAs and in response to observed clinical responses to the therapy. Normal target values for DHA level in plasma range from about 10 to 30 μ g of DHA per ml of plasma. Once normalized or

supernormalized, level(s) of the circulating DHA have been achieved and/or desired clinical effects are observed, the daily dose of oil(s) may be modified to maintain the circulating DHA at a desirable level.

As noted above, in order to treat certain disorders, it may be desirable 5 to raise the level of DHA in target tissue(s) and /or level of circulating DHA in the blood to 2 to 5 times normal levels. The levels of circulating DHA, therefore, can be raised to about 120-150 μ g/ml.

Monitoring DHA levels and dosages are also described in patent publications WO 94/28913 and WO 96/40106, the texts of which patent publications 10 are herein incorporated by reference.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

We Claim:

1. A therapeutic composition comprising highly unsaturated fatty acids (HUFAs) in an amount and form sufficient to produce normal or supernormal levels of one or more of the respective HUFAs in one or more tissues of an individual in need thereof upon administration of the composition.
5
2. The therapeutic composition of claim 1, wherein tissue level of one or more HUFA are raised to over twice normal in a target tissue.
3. The therapeutic composition of any preceding claim, wherein tissue level of docosahexaenoic acid (DHA) is raised to over twice normal in the target
10 tissue.
4. The therapeutic composition of any preceding claim, wherein the composition contains docosahexaenoic acid (DHA) in an amount equal to or greater than three times the molar amount of eicosahexaenoic acid (EPA) in the composition.
- 15 5. The therapeutic composition of claim 4, wherein the DHA:EPA ratio is at least 5.0.
6. The therapeutic composition of any preceding claim, wherein EPA makes up no more than 20% of the fatty acids or fatty acid residues in the composition.
- 20 7. The therapeutic composition of any preceding claim, wherein EPA makes up no more than 10% of the fatty acids or fatty acid residues in the composition.
8. The therapeutic composition of any preceding claim, wherein EPA makes up no more than 5% of the fatty acids or fatty acid residues in the composition.
25
9. The therapeutic composition of any preceding claim, wherein EPA makes up no more than 1% of the polyunsaturated fatty acids in the composition.

10. The therapeutic composition of any preceding claim, wherein the individual is a mammal, especially a human, suffering from a disease selected from the group consisting of cystic fibrosis, multiple sclerosis, cerebral palsy, amyotrophic lateral sclerosis, phenylketonuria, and neurological disorders, including
5 certain neurodegenerative diseases and psychiatric disorders.

11. The therapeutic composition of any preceding claim, wherein the levels of one or more of the respective HUFAs are normalized or supernormalized in membranes of the individual.

12. The therapeutic composition of any preceding claim, wherein the
10 HUFAs are in a readily absorbable form, such that the dose of HUFA required to achieve normal or super normal levels in the individual is lower than the dose required for HUFA-containing triglycerides.

13. The therapeutic composition of any preceding claim, wherein the HUFAs are predominately in the form of glycerol esters.

15 14. The therapeutic composition of any preceding claim, wherein at least 70% of the HUFAs are esterified to glycerol.

15. The therapeutic composition of any preceding claim, wherein the HUFAs are predominately in the form of diglycerides and/or monoglycerides.

20 16. Preparation of a therapeutic composition comprising highly unsaturated fatty acids (HUFAs) in an amount and form sufficient to produce normal or supernormal levels of the respective HUFAs in an individual in need thereof upon administration of the composition.

25 17. The preparation according to claim 16, wherein the composition also contains one or more enzymes which are suitable for consumption by mammals and that can catalyze hydrolysis of glycerol esters after administration to the individual.

18. The preparation according to claim 16 or 17, wherein the enzyme is incubated with HUFA-containing triglycerides before administration of the composition to the individual.

19. The preparation according to any of claims 16-18, wherein the
5 HUFAs are predominately in the form of glycerol esters.

20. The preparation according to any of claims 16-19, wherein all or a part of the glycerol esters are formed by glycerolysis of triglycerides.

21. The preparation according to any of claims 16-20, wherein the reaction mixture for glycerolysis of triglycerides also contains one or more free
10 HUFAs.

22. The preparation according to any of claims 16-21, wherein all or a part of the glycerol esters are formed by synthesis from glycerol and free HUFA.

23. The preparation according to any of claims 16-22, wherein formation of the glycerol esters is catalyzed by one or more enzymes.

15 24. The preparation according to any of claims 16-23, wherein the composition is formulated for administration to the individual of 10-20 grams/day of one or more HUFAs.

25. The preparation according to any of claims 16-24, wherein the HUFA consists essentially of DHA.

20 26. A method for treatment of pathologies which are characterized by abnormally low levels of docosahexaenoic acid in the membrane lipids of characteristic tissues using a preparation of an absorbable form of docosahexaenoic acid (DHA) with less than 1 part eicosapentaenoic acid (EPA) to 3 parts DHA, such that the oral consumption of such a preparation leads to the normalization or 25 supernormalization of the tissue DHA levels and reduction of pathologic symptoms.

27. The method of claim 26, wherein the pathology is a disorder selected from the group consisting of multiple sclerosis, cerebral palsy, amyotrophic lateral

sclerosis, phenylketonuria, and neurological disorders, including certain neurodegenerative diseases and psychiatric disorders.

28. The method of claim 26, wherein the pathology is cystic fibrosis.
29. The method of claim 26, wherein the preparation comprises a therapeutically effective amount of a DHA-rich triglyceride oil, wherein the triglyceride is hydrolyzed or pre-digested prior to consumption to yield DHA completely in the form of monoglycerides, completely in the form of diglycerides, completely in the form of free fatty acids, or any combinations or mixtures thereof.

30. The method of claim 26, wherein the preparation contains less than
10 20% EPA.

31. The method of claim 29, further wherein the hydrolysis or pre-digestion is performed on an oil in which the DHA content before hydrolysis or pre-digestion is above 20%.

32. The method of claim 29, further wherein the hydrolysis or pre-
15 digestion is performed on an oil in which the DHA content before hydrolysis or pre-
digestion is over 40% DHA.

33. The method of claim 32, wherein the oil is produced by an alga.

34. The method of claim 33, wherein the oil is produced by the alga *Cryptocodinium cohnii*.

20 35. The method of claim 32, wherein the oil is produced by fungi.

36. The method of claim 32, wherein the oil is produced by fungi of the chytrid family.

37. The method of claim 36, wherein the oil is produced by fungi selected from *Thraustochytrium*, *Schizochytrium*, and *Ulkenia*.